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Eco-friendly management of early blight of tomato (*Lycopersicon esculentum* Mill.)

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Abstract
Tomato crop is attacked by number of fungi, bacterial and viral diseases. Disease is major constrains in economic crop production as they inflict heavy losses. Among all the fungal diseases early blight of tomato (*Alternaria solani*) has currently become one of the most important disease of all tomato varieties. Therefore present investigation was under taken for the management of early blight using cow urine, cow dung, vermi wash, bio-agents, eucalyptus oil and fungicide (treated as check) for their efficacy under *in vivo* treatments were evaluated on different growth and yield parameters of tomato crop under field conditions at Prayagraj during Rabi season 2020-2021. Maximum Plant height @90DAT (60.58cm), maximum number of branches @90DAT (60.58). *Trichoderma harzianum* was found effective against reducing the disease intensity @90DAT (31.50%) Followed by cow urine, vermiwash, eucalyptus oil compared to Azoxystrobin (check) and control. The maximum yield was found with *Trichoderma harzianum* (16.01t/ha), eucalyptus oil (15.53t/ha), cow dung (15.45t/ha) as compared to Azoxystrobin (treated check) (18.83t/ha) and control (5.35t/ha).

Keywords: *Alternaria solani*, cow urine, cow dung, eucalyptus oil, fungicide *Trichoderma harzianum*, vermiwash

Introduction
Tomato (*Lycopersicon esculentum* Mill.) is one of the most popular fruit vegetable crop grown throughout the world. It is considered as "Protective food", because of its special nutritive values and its wide spread cultivation. Tomato crop is affected by several diseases caused by fungi, bacteria, viruses and a biotic factors. Among the biotic causes, fungi are most important which induce the several major diseases.

Alternaria solani (Ellis and Martin) is a causal agent of early blight on tomato (Madden *et al*., 1978) [5]. The genus *Alternaria* is soil born pathogen and many species of this genus including *Alternaria solani* are known to be plant pathogen (Alhussaen, 2012) [2]. The *Alternaria solani* is from phylum Ascomycota, class Othideomycetes, order Pleosporales and family Pleosporaceae (Alhussaen, 2012) [2]. *Alternaria solani* contains enzymes such as celluloses which degrade the host cell wall and also contain pectin methyl galacturonase which facilitate host colonization (Shahbazi *et al*., 2011) [14]. This disease affect crop production as they cause premature defoliation and result in heavy losses in production by reducing quality and quantity of fruit (Holm *et al*., 2003) [3].

Cow dung and cow urine which are capable of treating many diseases have several medicinal properties and are the best remedy to cure fungal and bacterial diseases. (Savita *et al.*, 2015) [12]. The prophylactic and preventive effects of cow by products can also be utilized to combat the detrimental effect of *A. solani*. Antagonistic property of cow dung and cow urine, in a ratio of 3:1 was determined Nene *et al*., (2002) [8] and it plays an important role in managing pathogens. Noor *et al.,* (2003) [9] reported the antagonistic effect of cow urine and confirmed that it can be used good and potential pesticide, determined antifungal activity of cow urine at various concentrations *viz*., 5, 10 and 15% against (*F. oxysporum*, *R. solani*, and *S. rolfsii*) that were purified from infected plants of both Fenugreek, Okra. Among all, cow urine @ 15% was found most effective.

Vermiwash, a collection of excretory products and mucus secretion of earthworms along with micronutrients from the soil organic molecules, stimulate the growth and yield of crops and can be used to cure diseases. Although work pertaining to biocidal activity of Vermiwash is there but no report is available practical application of Vermiwash to control plant diseases.

Azoxystrobin is a novel fungicide had found effective in lowering the disease severity throughout the growing period of crop (Pallavi *et al*., 2020) [11].

Materials and Methods

Experimental site: The experiment was conducted at the Department of Plant Pathology and Central Research Field, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj during Rabi season 2020-2021. The trail was laid out in Randomized Block Design with three replications. The various treatments included under study were as follows:

Collection of disease samples: Plants showing typical symptoms, in the field of standing crop *i.e*., the infected plant part of Tomato was selected. These disease plant materials were brought to the lab for further investigation.

Isolation of the pathogen

The pathogen was isolated from the disease infected plants and it was identified as the *Alternaria Solani*. Early blight of Tomato infected leaves were collected. The infected leaves dissected into small pieces (0.5cm^2) and surface sterilized was done with mercuric chloride (0.1%) for 15-30 seconds, rinsed with three changes of sterile distilled water to remove the disinfectant and blotted dry. The sterilized leaf pieces are placed (4 pieces/dish) on potato dextrose agar (PDA) medium in Petri dishes under aseptic conditions and incubated at 25 ^{0}C for 2 weeks. For obtaining sufficient quantity of inoculums, pure cultures was obtained by sub culturing. For this purpose, small bits of the fungus were taken at the tip of a sterilized needle and transferred aseptically to the centre of fresh PDA medium in Petri dishes. The dishes were incubated for 2 weeks at 25 °C in the dark.

Identification of the Pathogen

Morphological studies of the pathogen were conducted from pure culture. Spore suspension in sterilized distilled water was made from pure culture of the pathogen grown on PDA. One drop of the spore suspension was placed on a slide and morphological characters were noted with the help of microscope.

Maintenance of the culture

The fungus was sub cultured on PDA slants and allowed to grow at 27 ± 1 ºC for 15 days. Such slants were preserved in refrigerator at 5 ºC and sub cultured once fortnightly. This pure culture was used for further study.

Evaluation of various treatments *in vivo*

The treatments were sprayed and obeservations were noted down at 30 days interval after initiation of disease. Obeservations on disease severity of pathogen were recorded at 30, 60, 90 DAT

Disease severity scale of *Alternaria solani* early blight

Disease intensity was recorded as grades in five randomly selected plants in each plot at different time that is before spraying, 30 days after the first spray and 30 days after the second spray as per the scale mentioned by (Mayee and Datar 1986) [6].

Disease severity scale of *Alternaria solani* early blight

Grade	Disease severity description
0	No symptoms of disease on leaves
1	Small light brown spots scattered covering<=5% leaf area
2	Spots small brown, with concentric rings, covering 5.1 to 10% leaf area
3	Spots large brown, irregular with concentric rings, covering 10.1 to 25%leaf area
4	Large brown, irregular lesions with typical blight symptoms, covering 25.1to 50% leaf area
5	Large brown, irregular lesions with typical blight symptoms, covering more than 50%leaf area

Percent Disease Index (PDI) was calculated by using formula as given below (Wheeler, 1969).

$$PDI = \frac{\text{Sum of numerical disease ratings}}{\text{No. of plants observed X Maximum disease rating}} \times 100$$

Cost benefit ratio

Benefit cost ratio gross returns to the cost of cultivation, which can also be expressed as return per rupee invested. This index provides an estimate of the benefit a farmer derives from the expenditure in incurs in adopting a particular cropping system. The benefit cost ratio was calculated using the following formula

$$\text{B: C ratio} = \frac{\text{Net return (₹/ha)}}{\text{Total cost of cultivation (₹/ha)}}$$

Results and Discussion

Effect of treatments on plant growth characters of tomato

(Table 1) Observations were recorded at 90 DAT. Growth parameters included plant height (cm), no. of branches and no. of leaves. Of all the treatments T_2-*Trichoderma harzianum* showed maximum plant height at 90 DAT.

Maximum plant height was recorded in treatment with T_2 *Trichoderma harzianum* (60.58cm) which was superior over all the treatments, followed by T_3 *Pseudomonas fluorescence* (59.83cm), followed by T_5 Vermiwash T_7 (57.75cm), followed by T_1 Cow urine (59.66cm) and followed by T_6 eucalyptus oil (56.25cm) in comparison with T_0 control (53.16cm) and T_7 Azoxystrobin (93.83cm). All the treatments found statistically significant from T0 Control among all the treatments (T_0 and T_1, T_2 and T_3, T_3 and T_4, T_5 and T_4 and T_6; T_6 and T_7) were found non-significant to each other.

Maximum number of branches was recorded with T_2 *Trichoderma harzianum* (23.33) which was superior over all the treatments, followed by T_3 *Pseudomonas fluorescence* (22.75) followed by T_6 Eucalyptus oil (21.58) followed by T_5 vermiwash (20.58) followed by T_5 Cow dung (19.50) followed by T_1 cow urine (17.66) in comparison with T_0 control (15.91) and T_7 Azoxystrobin (22.00). All the treatments found statistically significant from T_0 Control among all the treatments (T_0 and T_1, T_2 and T_3, T_3 and T_4, T_5 and T_4 and T_6; T_6 and T_7) were found non-significant to each other.

Similar findings were also reported by Kadwal *et al*. (2019) [4] for *Trichoderma harzianum*. The probable reason for such findings may be that *Trichoderma* species interacts between the plant rhizosphere and soil micro-organisms plays an important role in plant growth and productivity. This resulted in good plant height and vegetative growth.

Effect of treatments on Disease intensity (%) of tomato

(Table 2) Disease intensity (%) observations were recorded at 90 DAT. Of all the treatments T_2-*Trichoderma harzianum* showed minimum disease intensity (%) at 90 DAT.

Minimum disease intensity (%) at 90 DAT was recorded with T_2 *Trichoderma harzianum* (31.50%), T_1 Cow urine (35.66%), T_5 Vermiwash (35.83%), T_3 *Pseudomonas fluorescence* (37.08%), T_6 Eucalyptus oil (37.33%), T_4 Cow dung (37.66%) followed by T_7 Azoxystrobin (28.41%) and minimum percent disease intensity (%) was recorded in T_0 control (38.66%). All the treatments were found statistically significant from T0 control among the treatments (T_0 and T_1; T_1 and T_2; T_3 and T_4; T_6 and T_7) are found non-significant to each other.

In the present studies minimum disease intensity (%) at 90 DAT was recorded with *Trichoderma harzianum* followed by cow urine and found effective over other. Present findings were similar to the findings of Selim *et al*. (2015) [13]. The probable reason for such findings in case of T*richoderma harzianum* may be that they releases the lytic enzymes in the rhizosphere, which catalyzes the damage of cell wall of target fungi. This resulted in inhibition of the pathogen thus resulted in better growth and good health of tomato plants.

Effect of treatments on root length (cm) and yield (t/ha)

(Table 3) Observations were recorded at harvest. Yield parameters include root length (cm) and yield (t/ha). Among all the treatments, maximum root length was recorded in the T_3 *Pseudomonas fluorescence* (16.25cm), T_2 *Trichoderma harzianum* (16.25cm), T_6 Eucalyptus oil (14.75cm), T_4 cow dung (14.41cm), T_5 Vermiwash (13.58cm) and T_1 Cow urine (11.66cm). All the above treatments are significant in comparison with T_0 control (11.66cm) and T_7 Azoxystrobin (15.41cm). All the treatments found significant to T_0, among the treatments (T_0 and T_1; T_1 and T_2 and T_3; T_2 and T_3 and T_4; T_3 and T_4 and T_5; T_5 and T_6; T_6 and T_7) were found non-significant to each other.

Similar findings also reported by Ahirwar *et al*. (2015) [1]. The probable reason is the inoculation of *Pseudomonas* PGPR with ACC deaminase activity having the potentiality to reduce ethylene concentration is one of the most effective and alternative strategies to induce tolerance in plants against various abiotic and biotic stressed condition where is directly attributed to increase in the root development in tomato plant.

Among all the treatments, maximum fruit yield (t/ha) was recorded in T_2 *Trichoderma harzianum* (16.01t/ha) which was superior over all the treatments, followed by T_6 eucalyptus oil (15.53t/ha), followed by T_4 Cow dung (15.45t/ha), followed by T_5 vermi wash (14.55t/ha), followed by T_3 *Pseudomonas fluorescence* (14.01 t/ha) and followed by T_1 cow urine (7.2 t/ha) as comparison to T_0 control (5.35 t/ha) and T_7 Azoxystrobin (18.23 t/ha).

Effect of treatments on economics of chilli

Highest B:C ratio (table 4) Shows in T_2 *Trichoderma harzianum* (3.03) followed by T_4 Cow dung (2.81) followed by T_3 *Pseudomonas fluorescens* (2.74) followed by T_6 Eucalyptus oil (2.72) followed by T_5 Vermiwash (2.71) followed by T_1 Cow urine (1.69) as compare to T_0 control (1.02) and Azoxystrobin (3.05).

Table 1: Effect of treatments on plant height (cm), number of branches per plant, number of leaves per plant at 90 DAT

Tr. No	Treatments	Plant height (cm)			90 DAT	
		30 DAT	60 DAT	90 DAT	Branches	Root length (cm) After harvesting
T_0	Control	26.91	40.50	53.16	15.91	11.66
T_1	Cow urine	29.83	43.66	59.66	17.66	12.08
T_2	*Trichoderma harzianum*	33.25	46.50	60.58	23.33	16.25
T_3	*Pseudomonas fluorescens*	31.08	44.83	59.83	22.75	16.91
T_4	Cow dung	27.25	43.00	55.83	19.50	14.41
T_5	vermiwash	28.66	43.08	57.75	20.58	13.58
T_6	Eucalyptus oil	27.33	43.58	56.25	21.58	14.75
T_7	Azoxystrobin	31.91	45.16	60.33	22.00	15.41
	F-test	S	S	S	S	S
	C.D (%)	2.80	2.11	2.53	1.87	3.23
	SEd±	1.301	0.986	1.23	0.874	1.505

Table 2: Effect of treatments on disease intensity (%) on tomato plant at different growth intervals

Tr. No	Treatments	Disease intensity (%)		
		30 DAT	60 DAT	90 DAT
T_0	Control	23.66	33.66	38.66
T_1	Cow urine	14.00	23.41	35.66
T_2	*Trichoderma harzianum*	12.41	22.58	31.50
T_3	*Pseudomonas fluorescens*	17.08	24.83	37.08
T_4	Cow dung	19.00	27.16	37.66
T_5	Vermi wash	15.75	27.16	35.83
T_6	Eucalyptus oil	15.91	27	37.33
T_7	Azoxystrobin	7.16	18.66	28.41
	F-test	S	S	S
	C.D (5%)	1.86	1.76	2.2
	SEd±	0.8660	0.820	1.031

Table 3: Effect of treatments on yield (t/ha) on tomato plant at harvest

Tr. No.	Treatments	Yield (t/ha)
T_0	Control	5.35
T_1	Cow urine	7.26
T_2	*Trichoderma harzianum*	16.01
T_3	*Pseudomonas fluorescens*	14.01
T_4	Cow dung	15.45
T_5	Vermiwash	14.55
T_6	Eucalyptus oil	15.53
T_7	Azoxystrobin	18.23
	F-test	S
	C.D (5%)	0.73
	SEd±	0.340

Table 4: Effect of treatments on economics of tomato

Tr. No	Treatments	Cost of cultivation (Rs.)	Gross returns (Rs.)	Net returns (Rs.)	B:C Ratio
T_0	Control	100.120	102,334	2,214	1.02
T_1	Cow urine	105,970	179,066	73,097	1.69
T_2	*Trichoderma harzianum*	103,320	312,600	2,07,880	3.03
T_3	*Pseudomonas fluorescens*	103,320	282,600	1,77,880	2.74
T_4	Cow dung	115,520	324,533	2,19,188	2.81
T_5	Vermiwash	107,195	290,400	1,83,205	2.71
T_6	Eucalyptus oil	114,330	310,600	1,90,380	2.72
T_7	Azoxystrobin	121,220	369,600	1,80,714	3.05

Conclusion

It is concluded that using chemicals up to certain boundary is beneficial but using in continuously is harmful for nature and human kind. Present work is ecofriendly approach they are having potential to inhibit the growth of fungus i.e., disease reduction. The present research concludes that among all the treatments *Trichoderma harzianum* showed the best results in inhibiting the growth of the fungus *Alternaria solani* as well as significant in the plant growth characters and yield. By conducting further field trials by using bioagents would help farmers to recommend the usage of *Trichoderma* which is economical and ecofriendly.

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